Policy Title :		
ABO Cell Grouping (Reverse Grouping)		
Department	Index No.	Scope
Laboratory & Blood Bank	LAB-062	All Laboratory Staff
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01. Policy:

The ABO system is the only blood group system where individuals older than 2-6 months predictably produce antibodies to antigens they are lacking. Direct agglutination of A1, B cells indicate the presence of the appropriate ABO antibody. Failure of the patient serum to the agglutinate the reagent cells indicates the absence of the corresponding antibodies. The pattern of the agglutination can therefore be interpreted and used in determining the patient's ABO group.

02. Definition :

N/A

03. Purpose :

Serum grouping tests, employing reagent red cells of known ABO groups, are used as a confirmation to cell typing procedures performed with antisera. Because of the importance of the ABO groups in transfusion, it is essential that serum and cell typing be performed on all specimens.

04. Procedure :

1. **Tube method technique:**

1. 1. **Specimen Requirements**

plasma Testing should be completed within 24 hours.

1. 2. **Materials**

1.2.1. Glass test tubes

1.2.2. Blood Bank pipettes

1.2.3. Calibrated serologic centrifuge

1.2.4. Microscope and slides

1. 3. **Reagents**

1.3.1. Reagent cells A1

1.3.2. Reagent cells B

1.3.3. Reagent cells O

1. 4. **Method**

1.4.1. Label three tubes with the patient identification and the reference cells to be tested: A1, B and O .

1.4.2. Using plastic pipettes, place two drops of the serum to be tested in each tube. (one drop of serum minimum)

1.4.3. Add one drop well mixed reference cells (A1, B&O) to the appropriate tube.

1.4.4. Mix by gently shaking. Tubes may be incubated 5-60 minutes at room temperature to enhance reactions of weakly reactive antibodies.

1.4.5. Centrifuge according to the optimum saline spin time listed for the centrifuge.

1.4.6. Examine macroscopically and microscopically

1.4.7. Record results in blood grouping register immediately.

1. 5. **Quality Control:**

Reagents are to be tested with appropriate positive and negative controls daily and the results recorded.

1. 6. **Reporting Results:**

1.6.1. **Interpretation**

- A positive test is indicated by agglutination of any of the reference cells. This shows the presence of an antibody corresponding to an antigen on those cells.
- A negative test shows no agglutination.
- Reactions should show the following patterns:

Blood Group	A1	B	O
O	+	+	-
A	-	+	-
B	+	-	-
AB	-	-	-

1.6.2. **Reporting Results**

1. 7. **Limitations:**

1.7. 1. Reagent A1 and B cells possess blood group antigens other than A and B. On occasion a patient's serum will contain a cold reactive, saline phase agglutinin that may react with one of these additional antigens. Reactions due to non-ABO related antibody may interfere with reverse serum grouping tests.

1.7. 2. A negative result may be obtained with one or more of the reagent red cells if the test serum's antibody concentration is too low to be detected by the above methods. This may be observed in serum of patients who are elderly or are less than 6 months old.

1.7. 3. Umbilical serum, which may contain maternal anti-A and or anti-B, will not give reliable grouping results.

1.7. 4. Over centrifugation and under centrifugation must be avoided. These will lead to a false positive and negative reactions and therefore incorrect interpretations.

1. 8. **Discrepancies between forward and reverse grouping may be caused by:**

1.8. 1. An atypical antibody in the patient's serum which corresponds to an antigen other than ABO on the reference cells.

1.8. 2. In cases of hypo-gammaglobulinemia the immunoglobulin deficiency in the patient may result in the inaccurate reverse grouping.

1.8. 3. Patient is a weak sub-group of A (or B). Incubation for 30 minutes at room temperatures may reveal weak positives.

1.8. 4. Leukemia may cause Ag determinants of ABO to fluctuate.

1.8. 5. Cord ABO determinants are not always well developed at birth.

1.8. 6. Recent previous transfusions of blood not of the same ABO type.

1.8. 7. Spontaneous aggregation of cells due to rouleaux, non specific cold agglutinins or bacterial contamination.

2. **Gel Micro typing System**

2.1. **Materials**

2.1.1. ID-Centrifuge

2.1.2. ID-Incubator

2.1.3. ID-Working table

2.1.4. ID-Dispenser

2.1.5. ID-Pipette FP-4

2.1.6. ID-Disposable tips

2.2. **Reagents**

2.2.1. ID-Diluent 1(Bromelin Solution)

2.2.2. ID-DiaCell A1

2.2.3. ID-DiaCell B

2.3. **Micro typing cards**

ABO + reverse group

2.4. **Sample**

Serum or plasma.

2.5. **Test procedure**

2.5.1. Allow all reagents to reach room temperature before use.

2.5.2. Identify the ID- micro typing card with the patient name and number. Remove the aluminum foil.

2.5.3. Add 50µl of ID-DiaCell A1 to micro tube A1.

2.5.4. Add 50µl of ID-DiaCell B to micro tube B.

2.5.5. Add 50µl of test serum or plasma to micro tubes A1 and B.

2.5.6. Centrifuge the micro typing card for 10 minutes in the ID-centrifuge.

2.5.7. Interpret the result.

3. **Interpretation**

Result can only be accurately interpreted if the control "clt" micro tube gives a negative reaction. A positive reaction may indicate the presence of autoantibody.

Blood Group	A1 cell	B cell
O	4-5	3-4
A	0	3-4
B	3-4	0
AB	0	0

4. **TANGO OPTIMO ANALYZER METHOD**

4.1. **Materials & reagents:**

- N rack of the Tango Optimo machine.

- Calibrated serologic centrifuge.
- Erytype™ S - ABO Donor.
- Bromelin for Erytype™(concentrate) .
- Erytype A1,A2, B and O cells.

4.2. **Sample:**

EDTA.anticoagulated blood sample after good centrifugation

4.3. **Test procedure:**

- Centrifuge Patients& donor blood sample & put them in (N) Rack without cups.
- Load Erytype™ S - ABO Donor plates.
- Load Erytype A1,A2, B and O cells.
- Open the sample door & put the (N) Rack in its correct place.
- Push the Rack button to select the desired rack on the screen.
- Press manual entry button &write the ID number of patients or donors samples.
- Press (ABO)button to select the test &close the sample door.
- Press accepted entry button
- Press start button to start the test.
- After the end of the test validate the results.
- Print out the results.

4.4. **Quality Control:**

Control Set QC contain test erythrocytes with defined antigen pattern for ABO,Rh(D), phenotyping(RhCEce and Kell) as well as isoagglutinins for reverse typing,

- Group O Neg
- Group AB Pos
- Group A neg
- Group O Pos
- Solidscreen 11-Control is used for Antibody screen QC

5. **Quality Control**

Reagents are to be tested with appropriate positive and negative controls daily and the results recorded. See "Daily Quality Control" for procedure.

6. **Reporting Results**

6.1. **Interpretation**

Agglutination of the red blood cells in the presence of the antiserum indicates the presence of the corresponding antigen. Likewise, no agglutination indicates the corresponding antigen is not demonstrable.

Reactions should show the following patterns:

Cell A1	Cell A2	Cell B	ABO Group Interpretation
+	+	+	**O**
-	-	+	**A**
+	+	-	**B**
-	-	-	**AB**

7. ORTHO VISION ANALYZER METHOD

7.1. **Materials & reagents:**

- antiA/antiB/AntiD ORTHO BIOVUE System cards
- Affirmagen (A1 and B cells) 3.5+/- 0.5% ORTHO BIOVUE System
- ORTHO BLISS
- VITORS 7% BSA

7.2. **Sample:**

- EDTA.anticoagulated blood sample after good centrifugation

7.3. **Test procedure:**

- Centrifuge Patients& donor blood sample & put them in a blue Rack without cups.
- Load antiA/antiB/AntiD ORTHO BIOVUE System cards
- Load Affirmagen (A1 and B cells) 3.5+/- 0.5% ORTHO BIOVUE System
- Load ORTHO BLISS and VITORS 7% BSA
- At the Graphical User Interface (GUI), touch Samples.
- Access the Samples screen by touching the Samples button on the menu bar or anywhere within the dark blue area of the Samples section.
- The GUI will display a graphical representation of the patient sample load positions. Touch the desired load position to be presented in the ORTHO VISION® Max Analyzer's load station. Use the Switch Load Station button to navigate between Load Stations 1 and 2.

- Touch Load/Unload. The software will display a wizard which prompts the operator to open the Load Station door.
- When the door is opened, the load station will present the quadrant that was selected.
- Load the samples
- Back at the GUI, touch a new load position and perform the same steps for any other trays to be loaded into the ORTHO VISION® Max Analyzer.
- When done loading all the trays, close the Load Station door.
- To assign a sample ID manually, touch the icon that corresponds to the sample tube.
- On the GUI, touch Assign to Position. The software opens the Assign to Position wizard.
- Enter the barcoded sample ID, which is displayed on the sample tube
- Touch Verify Sample button.
- Close the Load Station door.
- To assign profiles manually, touch the yellow icon that corresponds to the desired sample tube.
- Touch Create Order
- Verify the sample ID number on the screen that appears
- Touch Assign Profile, which is highlighted in red.
- Touch the profiles required for this particular sample for example Blood grouping ABO-Rh D
- NOTE: Profiles displayed have been previously created on the analyzer during set up
- Touch Save and Start.

7.4. **Reporting Results**

- **For the indoor patients:**

  The Blood bank technician registers the results in a special blood grouping register for patient's band writes the blood grouping result on the blood grouping request signs and writes the date and time.

- **For the outdoor patients:**

  The Blood bank technician enters the results via **the Medical Plus interface.**

- **For the donors:**

  The Blood bank technician registers the results in a special blood grouping register for donor

05. Responsibilities :

All laboratory of Al-Qunfudah General Hospital.

06. Equipment & Forms

06.1. Blood Grouping Book

07. Attachment :

07.1. NA

08. Reference

08.1. The Technical manual of the American Association of Blood Banks.

Preparation , Reviewing & Approval Box

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